isopropylmalate dehydrogenase
activity. The isolation of this mutant has been described by Gleeson et al. (1984).

(52) LR9 (CBS N.degree. 7172) is an auxotrophic derivative of H. polymorpha ATCC 34438, lacking orotidine 5'-decarboxylase activity.

(53) For the isolation, all procedures were carried out at 30.degree. C. instead of 37.degree. C., which is the optimal temperature for growth of this yeast. Yeast cells were mutagenized with 3% ethylmethanesulphonate for 2 hr (Fink, 1970). The reaction was stopped with 6% sodium thiosulphate (final concentration) and the solution was incubated for another 10 min. Mutagenized cells were then washed once with H.sub.2 O and incubated for 2 days on YEPD or
YNB supplemented with uracil for segregation and enrichment of uracil-auxotrophs followed by a 15 hr cultivation on MM without nitrogen source. Finally a nystatin enrichment was employed for 12 hr on MM with a concentration of 10 .mu.g antibiotic per ml. The treated cells were plated on YNB plates containing 200 .mu.g uracil per ml and 0.8 mg 5-fluoroorotic acid (Boeke et al., 1984). Usually 10.sup.6 cells were plated on a single